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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Supplementary Appendix

Genome-wide association study of survival from sepsis due to pneumonia: an observational cohort study

Rautanen A et al.

Supplementary METHODS

Subjects

GenOSept and GAinS

Caucasian patients admitted to European intensive care units (ICUs) with sepsis due to community acquired pneumonia (CAP) or peritonitis were recruited through the ESICM/ECCRN GenOSept (Genetics of Sepsis and Septic Shock in Europe) consortium from 143 centres across 16 European countries between September 2005 and October 2009 (Table 1). Ethics approval was granted either nationally, for individual centres or both. Written, informed consent was obtained from all patients or a legal representative.

GenOSept Consortium members developed and tested a case report form (CRF). Variables recorded pertained to demographic, clinical and outcome data. A specific electronic case report form (eCRF) was developed by Lincoln, Paris, France, using software developed in collaboration with GenOSept consortium members. The database was password protected, allowing investigators to enter data into the eCRF online, and included audit trail capability for data entry and subsequent modifications. To minimise errors, logical range checks were in place so that the investigators would be alerted if an attempt was made to enter data values outside the expected ranges. Quality Assurance (QA) was performed by five members of the GenOSept Consortium, who systematically reviewed all data. Data queries (DQs) were generated within the eCRF for missing or erroneous data, and, where necessary, were sent electronically to the relevant investigators for action, with a response rate by the investigators of more than 90%. All eCRFs were reviewed by experienced critical care physicians. Where the patient's eligibility for inclusion in the relevant cohort was unclear, clarification was sought from the investigators. Regular QA reports were provided to the GenOSept Management Committee for review; the National Investigators were contacted regarding quality issues if necessary.

Diagnosis of sepsis was based on the International Consensus Criteria[1]. Inclusion criteria were adult patients (≥18 years) admitted to a High Dependency Unit or ICU with CAP or peritonitis. Diagnosis of CAP was defined as a febrile illness associated with cough, sputum production, breathlessness, leukocytosis and radiological features of pneumonia acquired in the community or within less than 2 days of hospital admission[2]. Exclusion criteria were: pregnancy, an advanced directive to withhold or withdraw life sustaining treatment, admission for palliative care only and immune-compromise. Specific data were recorded in the eCRF to allow calculation of APACHE II scores[3]. Microbiological investigations were performed according to local policies and practices. Investigators recorded microbiological findings, including the organism(s) isolated, the source of the organism and the use of serological methods in the eCRF. Death or survival was recorded at 28 days from ICU admission, ICU discharge, hospital discharge and 6 months from ICU admission.

Blood samples were collected in 10 ml EDTA blood tubes and stored at -20°C until extraction. DNA was extracted from whole blood in centres in London, Paris and Munich. In London and Munich, extraction was performed using a salting out procedure[4].

In Paris, genomic DNA was extracted and purified from EDTA-stabilized whole blood samples using the MagNA Pure Compact Instrument with the MagNA Pure Compact DNA Isolation Kit for 1 ml volume (Roche, Mannheim, Germany) according to manufacturer recommendations. All GenOSept DNA samples underwent a strict quality check for concentration and integrity analyzed by agarose gel electrophoresis prior to genotyping. Some of the GAinS samples were extracted using Maxwell 16 Blood DNA Purification kits (Promega UK Ltd) with the Maxwell 16 instrument (Promega UK Ltd) at the Wellcome Trust Centre for Human Genetics, University of Oxford.

VASST

Caucasian patients were recruited between July 2001 and April 2006 as described before[5]. DNA was extracted from the buffy coat of discarded blood samples, collected for plasma analysis, using the QIAamp DNA maxi kit (Qiagen, Mississauga, ON, Canada). The research ethics board at the coordinating centre (University of British Columbia) approved the genetic analysis (approval number, H02-50076). Written consent was obtained from all participants or their authorised representative.

PROWESS

Caucasian patients were recruited between July 1998 and June 2000 as described before[6,7]. DNA was extracted from Whatman FTA™ blood spot cards, collected initially for analysis of the Factor V Leiden polymorphism. The ethics board at each study center approved the original trial protocol and written consent was obtained from all participants or their authorised representative. The Bioethics committees determined that no additional consent was necessary for further genetic studies on the anonymised samples.

Genome-wide genotyping and quality control

Genotyping of the GenOSept DNA collection was performed by one of the partners in the GenOSept Consortium (Munich, Germany) using the Affymetrix 5.0 SNP array. The additional GAinS sample set was genotyped using the Illumina Human OmniExpress Bead chip at the Wellcome Trust Centre for Human Genetics genotyping core facility, University of Oxford. The VASST and PROWESS sample sets were both genotyped using the Illumina Human 1M-Duo BeadChip array. Genotyping data of each cohort was cleaned in Oxford by removing poorly performing samples based on the following quality control (QC) criteria: call rate (<98%), extreme heterozygosity (>3 standard deviations from the mean), relatedness (PI_HAT (proportion identity by descent) > 0.2), population outliers (see example in Supplementary Figure 5), and gender discrepancies. See Supplementary Table 2 for a summary of excluded individuals in each sample collection. Only individuals with lung or abdomen as a source of infection and the patients for whom 28-day outcome was known were included in the analysis – also the number of phenotype exclusions based on this criteria are shown in Supplementary Table 2 and Figure 1.The following criteria were used to exclude unreliable SNPs: minor allele frequency (MAF) (<0.01), Hardy-Weinberg equilibrium ($P<1x10^{-10}$), and SNP call rate (<98% if MAF>0.05; <99% if MAF < 0.05). All these data cleaning steps were performed using PLINK[8]. After SNP QC, 354,483 (GenOSept), 644,775 (GAinS), 936,437 (VASST), and 934,810 (PROWESS) autosomal SNPs remained for imputation.

Imputation

The GWAS data sets (GenOSept, GAinS, VASST and PROWESS) were imputed separately by first pre-phasing the genotypes using SHAPEIT[9] before imputation using IMPUTE2[10]. The 1000 Genomes phase 1 (integrated January 2012) data version was used as a reference panel. Insertion deletion polymorphisms were removed; only SNPs were included in the analysis.

Statistical power

Statistical power to detect an association with 28-day survival from sepsis due to pneumonia with a conventional genome-wide significance p-value threshold of $5x10^{-8}$, varying odds ratios and minor allele frequencies, was calculated using R and is presented in Supplementary Figure 1. To select the SNPs to be genotyped in the final cohort, we used a commonly used p-value threshold of $< 1x10^{-5}$ for suggestive evidence of association in the discovery cohort. Statistical power with this less stringent p-value threshold in the discovery cohorts is shown in Supplementary Figure 1d.

Statistical analyses of the genome-wide data sets

All imputed data sets were analysed separately using SNPTEST2[11], taking into account the imputed genotype uncertainty (frequentist score test), and using age and the first four MDS components (generated in the QCd patient data exclusively; see Supplementary Figure 5b) as covariates in logistic regression with the additive model of association. These covariates were included in the model as age is known to be a strong determinant of mortality and the first four MDS dimensions were found to form distinct groups when study individuals were labelled by country (Supplementary Figure 5b). Because GenOSept and the additional GAinS discovery data sets were recruited using the same protocol, imputed genotypes were analysed together using SNPTEST2 before the meta-analysis.

The association results (OR and standard error) of each individual cohort were combined in a meta-analysis using PLINK. The more conservative random effects meta-analysis method was used instead of a fixed effects analysis as the latter tends to exaggerate p-values in the presence of heterogeneity[12]. Potentially unreliably imputed SNPs were filtered out based on minor allele frequency (<0.02), imputation info value from SNPTEST (< 0.8), and Hardy-Weinberg equilibrium ($P< 1x10^{-10}$). After these QC measures, 5,888,277 autosomal SNPs remained in the meta-analysis.

We performed a meta-analysis for 28-day survival in the predefined group of patients with sepsis originating from pneumonia (1553 individuals of whom 359 died).We also analysed the whole cohort of patients with sepsis originating from pneumonia or intra-abdominal infection (2534 individuals of whom 572 died within 28 days of ICU admission). The number of patients with intra-abdominal infections was too small for an adequately powered analysis. See Table 1 for the sample numbers in each individual cohort.

Additional genotyping and statistical analysis

Loci with P-values less than $1x10^{-5}$ in the meta-analysis of either pneumonia only or pneumonia and intra-abdominal infections combined, were genotyped using the Sequenom iPLEX MassArray system in the additional GAinS sample set of CAP and peritonitis sepsis patients combined (1002 patients of whom 174 died). Of these, 538 patients were diagnosed with CAP, of whom 106 patients died. Only SNPs with call rates> 90% and samples with call rates> 80% were included in the analysis. The additional set was analysed using PLINK with age as a covariate in logistic regression.

The most significant SNP after meta-analysis in patients with pneumonia, rs4957796, failed Sequenom genotyping and was therefore genotyped using High-Resolution Melting Curve Analysis (HRMA). PCR was performed with primers (Metabion) GGATGAGAGACTCAAATATCTATTGTT(F) and ATTATAGCCTCATTAGTTTAGAAATCCTAC(R). An unlabelled probe (Metabion) with a 3'-amino-C7 modification and sequence AATATCACATCATTGAAATTATTTGCTTTTAAGAA was used to improve genotyping resolution. Amplification was performed with an excess of the reverse primer and probe, to generate sufficient single-stranded product for the probe to anneal to. A 10µl reaction was used, containing 0.5µl 25ng/µl template DNA, 1µl 10x PCR buffer (Qiagen), 0.4µl 25mM $MgCl_2$ (Qiagen), 1µl dNTP mix (Bioline; 2mM of each dNTP), 1µl LC-Green Plus (Idaho Technology), 0.06µl HotStarTaq DNA polymerase (Qiagen), 1µl primer/probe mix (0.5µM forward primer, 5µM reverse primer and 5µM probe) and 5µl water. PCR steps involved an initial step at 95°C for 10 minutes, followed by 56 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds. Samples were then kept at 72°C for 5 minutes before cooling. A final melting step at 95°C for 1 minute was performed before allowing samples to cool to room temperature. High resolution melting was performed using a LightScanner (Idaho Technology) and melting was done from 50°C to 98°C at 0.1°C/sec. Melt curves were analysed using LightScanner software Call-IT 2.0 using derivative normalised melting plots between 62°C and 72°C.

96 samples were also Sanger sequenced in the forward and reverse direction using primers GGTGACTTTCTATAGCGTCTTTAAC (F) and GCCTCATTAGTTTAGAAATCCTACTT (R). All genotypes were identical between HRMA and sequencing. An example of a melting curve plot used to determine the rs4957796 genotype is shown in Supplementary Figure 6.

Because rs4957796 SNP was originally imputed, the discovery samples within the GenOSept cohort were subsequently directly genotyped using the HRMA method (we did not have access to the DNA samples collected within the VASST and PROWESS studies). Imputation concordance was calculated as the proportion of imputed genotypes that were consistent with direct genotyping. For this comparison the genotype with the highest probability was assigned as the imputed genotype. In the actual association analyses imputation probabilities rather than exact genotypes were used.

Time-to-event analysis

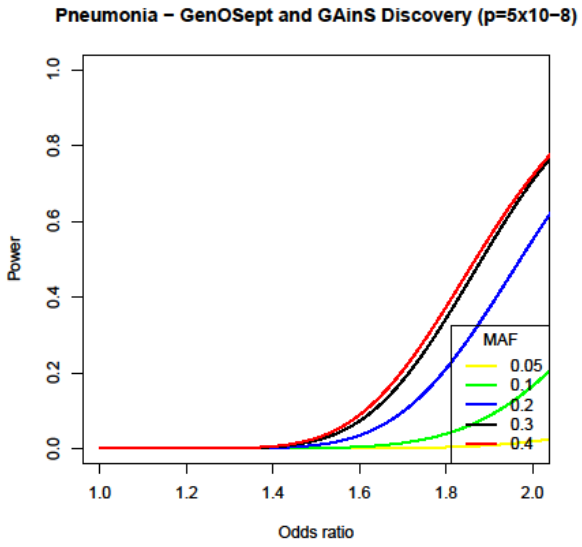
The follow-up of patients was continued for 6 months in GenOSept/GAinS, 3 months in VASST and 28 days in PROWESS. Cox regression models to evaluate the effect of genotype on survival time stratified by cohort and adjusted for age were performed using STATA v12. The proportionality of hazards was checked using the test on Schoenfeld residuals proposed by Grambsch and Therneau[13]. The survival analysis was repeated for time to death up to six months for GenOSept and GAinS cohorts. Owing to non-proportionality beyond 28-days, the

Cox model was extended with a term for interaction between genotype and time[14]. Survival curves were created using R.

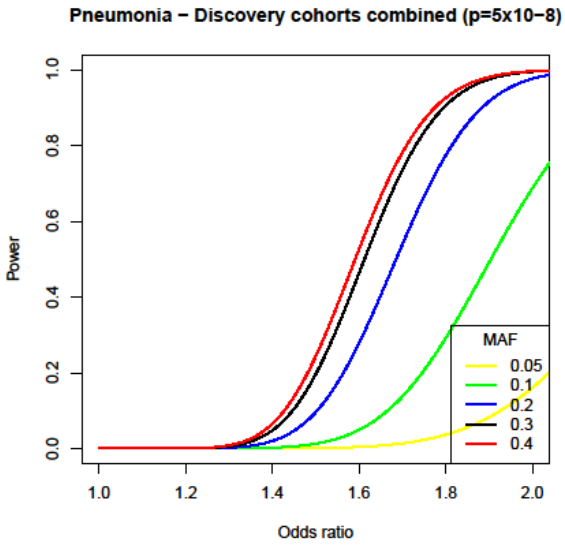
SUPPLEMENTARY FIGURES

Supplementary Figure 1. Statistical power to detect an association with 28-day survival from sepsis due to pneumonia. A) GenOSept discovery cohort, B) discovery cohorts combined, C) all four cohorts combined with a p-value threshold of $5x10^{-8}$, and D) discovery cohorts combined with a p-value threshold of $1x10^{-5}$ for a suggestive evidence of association are presented. Varying odds ratios and minor allele frequencies (MAF) are indicated.

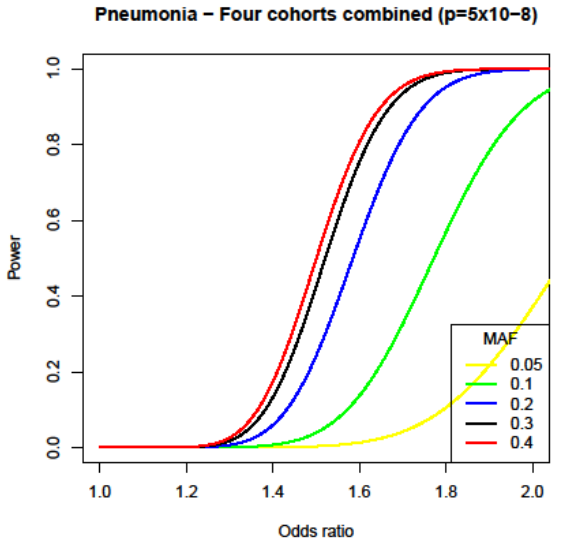
A)



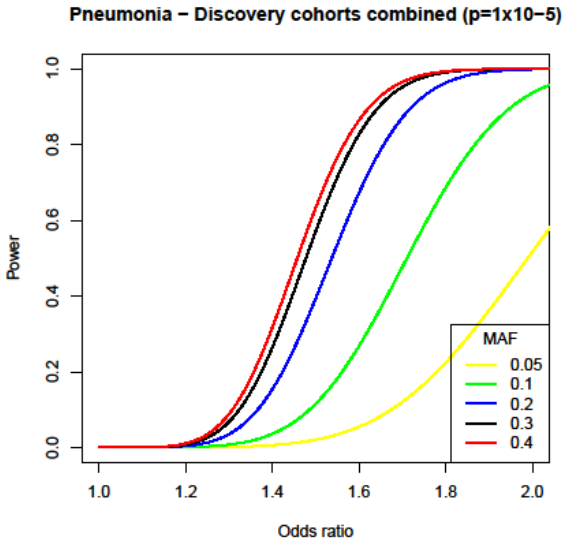
B)



C)

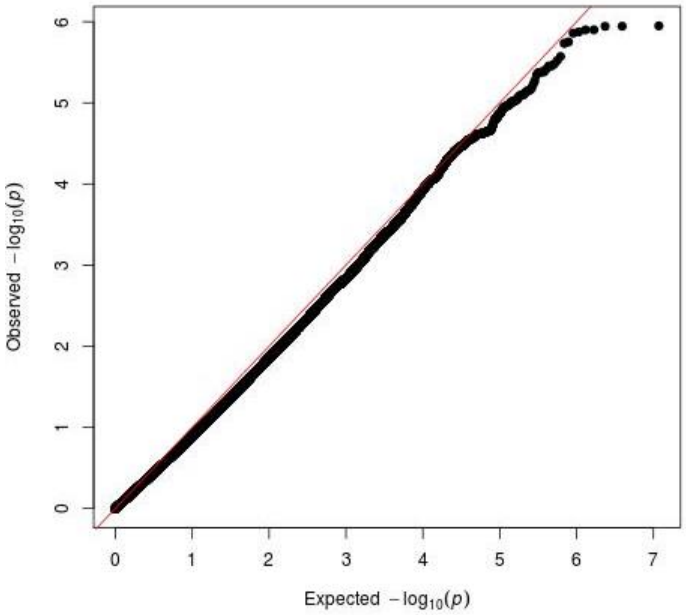


D)

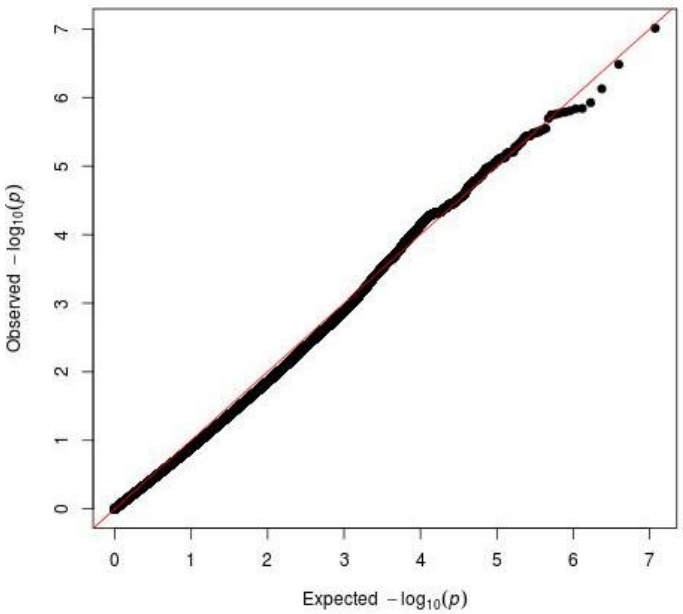


Supplementary Figure 2. QQ-plots of the meta-analysis of 28-day survival in patients with sepsis caused by (A) pneumonia or abdominal infection and by (B) pneumonia. The inflation factor lambda for pneumonia and abdominal infection and pneumonia only were 0.88 and 0.89, respectively.

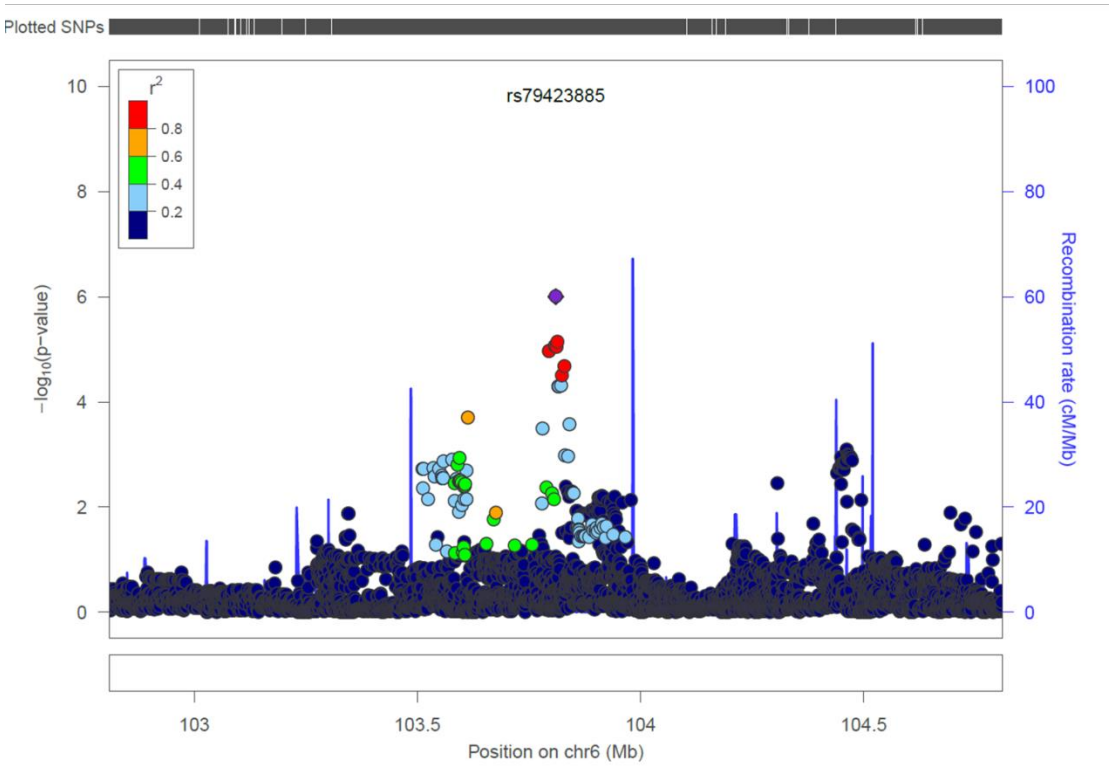
A)



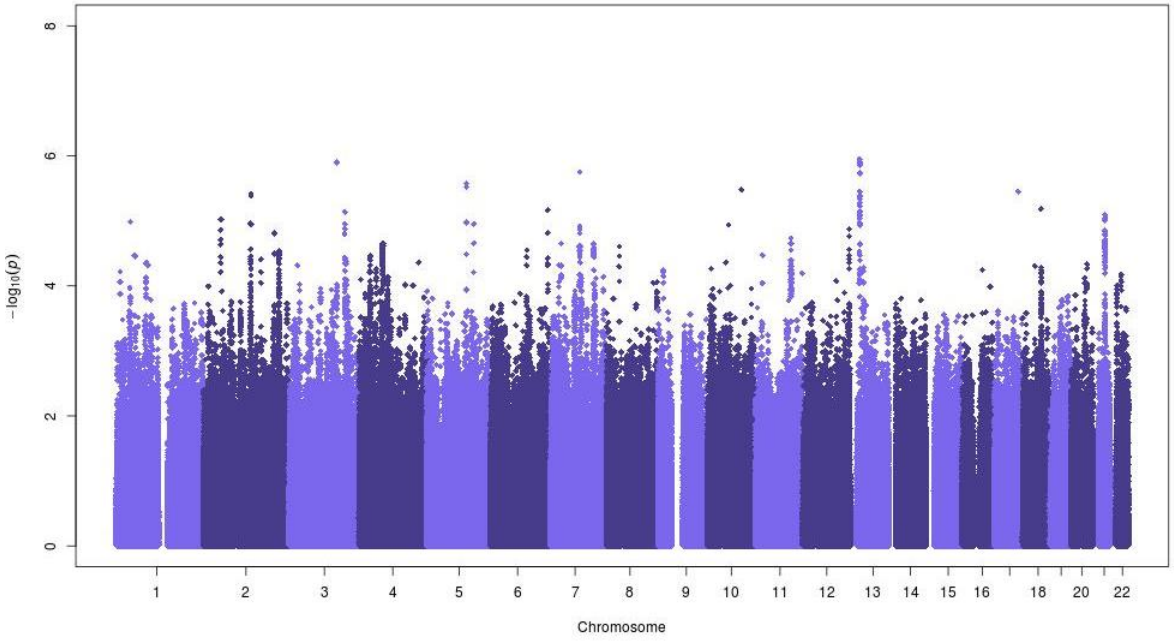
B)



Supplementary Figure 3. Regional association plot for the chromosome 6 locus (rs79423885) in the meta-analysis of 28-day outcome of patients with sepsis originating from pneumonia (additive model). Colours indicate the correlation (r^2 in CEU 1000 Genomes data) with the top SNP rs79423885. No functional elements for this locus have been identified from the ENCODE data (http://genome-euro.ucsc.edu/cgi-bin/hgTracks?db=hg19&position=chr6%3A103811003-103814490&hgsid=200149161_mTA8SVfMtELWtkoEWMvA5WQgMhdx last accessed 14.11.2014).

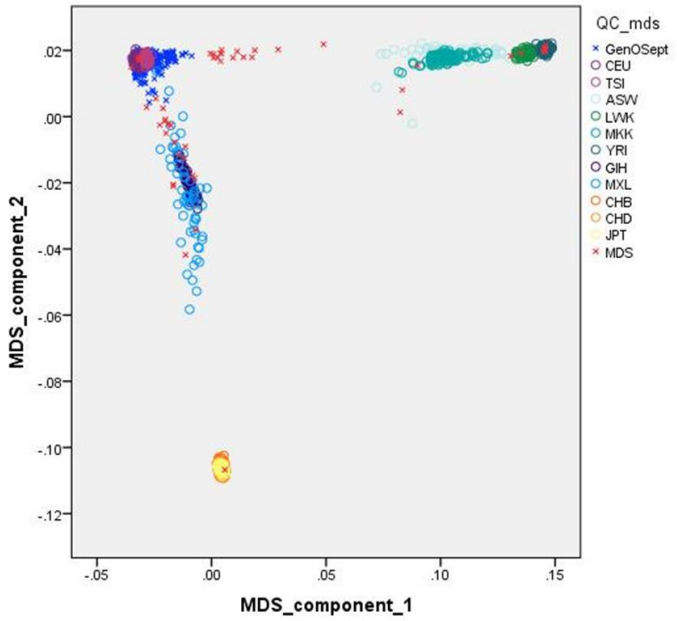


Supplementary Figure 4. Manhattan plot for the meta-analysis of 28-day outcome in patients with sepsis caused by pneumonia or abdominal infection (additive model). SNPs with minor allele frequency >2%, info value > 0.8, and Hardy-Weinberg equilibrium P> $1x10^{-10}$ are included (5,888,277 SNPs in total).

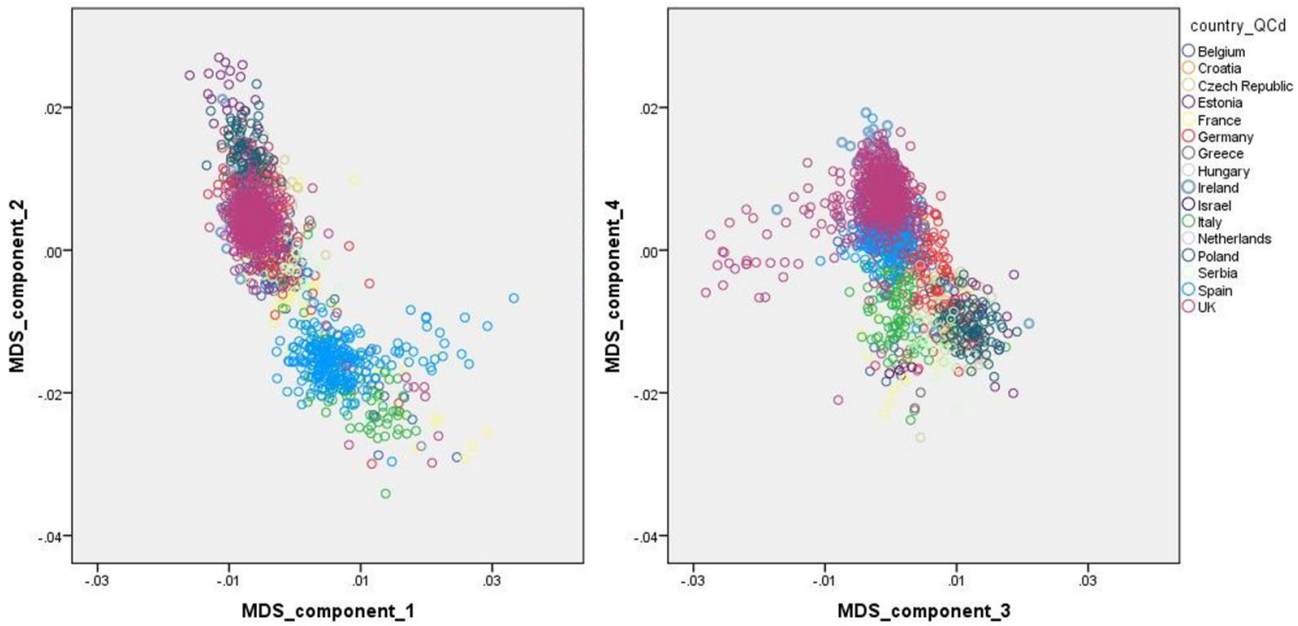


Supplementary Figure 5. Multidimensional scaling analysis to identify population outliers in the GenOSept cohort. A) Analysis performed together with the individuals from the 1000 Genomes Project. GenOSept samples (blue) and population outliers excluded from the analysis (red, labelled "MDS") are shown by crosses. All individuals from the 1000 Genomes Project are shown by circles and come from various populations; CEU: Utah residents with ancestry from northern and western Europe; TSI: Toscani in Italia; ASW: African ancestry in Southwest USA; LWK: Luhya in Webuye, Kenya; MKK: Maasai in Kinyawa, Kenya; YRI: Yoruba in Ibadan, Nigeria; GIH: Gujarati Indians in Houston, Texas; MXL: Mexican ancestry in Los Angeles, California; CHB: Han Chinese in Beijing, China; CHD: Chinese in Metropolitan Denver, Colorado; JPT: Japanese in Tokyo, Japan. B) Analysis performed only within the QCd set of GenoSept individuals with the country of origin highlighted in the plot. Left panel shows the first and second and the right panel third and fourth MDS dimensions.

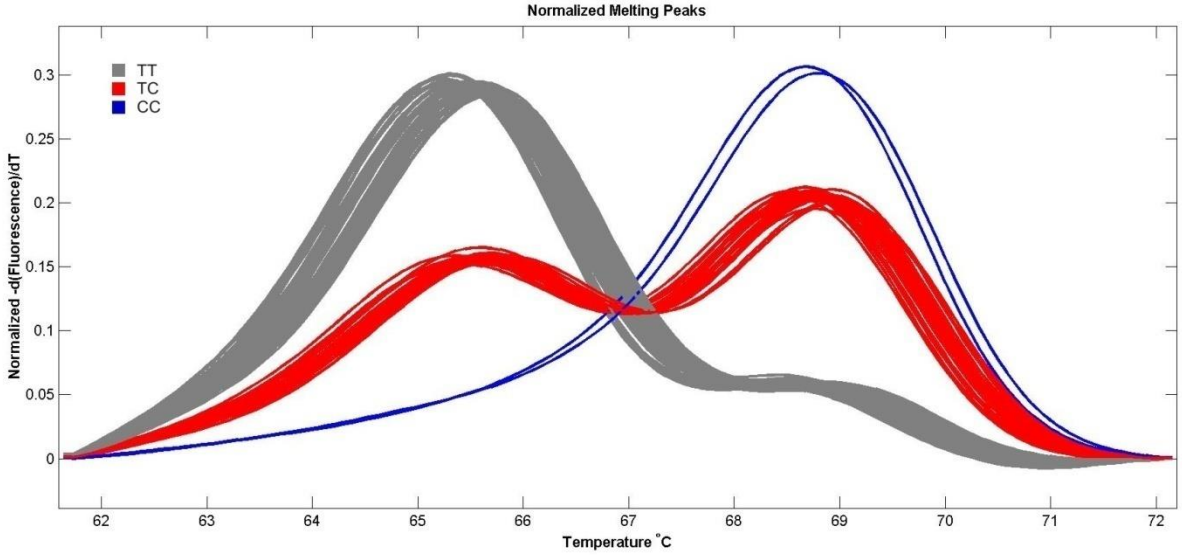
A)



B)



Supplementary Figure 6. HRMA genotyping plots for *FER* rs4957796 in the additional cohort. The figure illustrates melting curves for one 96-well plate. Grey, red and blue curves indicate TT, TC and CC genotypes respectively.



SUPPLEMENTARY TABLES

Supplementary Table 1. Definitions of sepsis, severe sepsis and septic shock.

Sepsis	Severe sepsis	Septic shock
The systemic response to infection. This systemic response is manifested by two or more of the following conditions: • Temperature >38^{0}C or <36^{0}C • Heart Rate >90 beats/min • Respiratory Rate >20 breaths/min or $PaCO_2$ <4.3 kPa or the need for mechanical ventilation • White blood cells >12,000 cells/mm^3, <4000 cells/mm^3, or >10% immature (band) forms	Sepsis associated with acute organ dysfunction, hypoperfusion or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to lactic acidosis, oliguria, or an acute alteration in mental status.	Sepsis with hypotension, despite adequate fluid resuscitation, along with the presence of perfusion abnormalities that may include, but are not limited to lactic acidosis, oliguria, or an acute alteration in mental status. Patients who are on inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured. Hypotension is defined as a systolic blood pressure <90mmHg or a reduction of >40mmHg from baseline in the absence of other causes of hypotension

Supplementary Table 2. Samples excluded from the genome-wide data sets.

Exclusion criteria	N of excluded samples in GenOSept (N=1770)	N of excluded samples in GAinS (N=288)	N of excluded samples in VASST (N=632)	N of excluded samples in PROWESS (N=720)
Call rate	68	8	2	52
Extreme heterozygosity (>3SD)	58	0	12	84
Population outliers	45	1	120	136
Discrepant gender	23	9	1	1
Duplicate samples	0	0	0	0
Relatedness (pi hat>0.2)	17	1	2	1
Duplicate samples between datasets	0	29	0	0
Total number* of samples removed based on above QC	187	46	123	193
Phenotype (28 day status not known or source of infection other than lung or abdominal)	58	1	148	120
Total samples removed	245	47	271	313
Samples remaining after QC	**1525**	**241**	**361**	**407**
Samples remaining after QC (pneumonia only)	**794**	**241**	**217**	**301**

*There may be more than one reason for excluding a sample

Supplementary Table 3. Top associations (loci with P < $1x10^{-5}$) after the genome-wide meta-analysis of 28-day survival among patients with sepsis caused by pneumonia (additive model). Results shown for GenOSept/GAinS (G), VASST (V), and PROWESS (P) discovery sets are based on imputed data (except where indicated), whereas the additional GAinS results are based on direct genotyping. Only the SNP with the most significant P-value per locus and those that were included in the additional GAinS genotyping are shown (more than one SNP per association peak was genotyped when possible). MAF=minor allele frequency; OR=odds ratio. SNPs highlighted in bold are those that show stronger evidence of association after adding the additional cohort in the combined analysis. NA denotes missing data. (* although p-value in the replication set is <0.05 the direction of the effect is in the opposite direction to that seen in the discovery set.)

Chr	Position	SNP id	Discovery Meta P	Discovery Meta OR	Directly genotyped	GenOSept P	GenOSept OR	GenOSept MAF cases	GenoSept MAF controls	VASST P	VASST OR	VASST MAF cases	VASST MAF controls	PROWESS P	PROWESS OR	PROWESS MAF cases	PROWESS MAF controls	Additional GAinS P	Additional GAinS OR	Additional GAinS cases MAF	Additional GAinS controls MAF	Combined P	Combined OR	Gene
1	82383883	rs146730869	5.3E-06	4.48	---	1.9E-04	4.65	0.048	0.016	0.077	3.92	0.034	0.010	0.053	4.54	0.025	0.011	NA	NA	NA	NA	NA	NA	*LPHN2*
2	134086271	rs10928450	9.0E-06	0.63	---	1.1E-04	0.58	0.174	0.262	0.072	0.63	0.169	0.238	0.120	0.73	0.240	0.290	NA	NA	NA	NA	NA	NA	*NCKAP5*
2	134091649	rs13392963	1.6E-05	0.63	VP	8.7E-05	0.57	0.170	0.259	0.073	0.63	0.169	0.238	0.210	0.78	0.250	0.291	0.352	1.06	0.267	0.254	4.4E-04	0.73	*NCKAP5*
2	201260365	rs78318430	2.0E-05	2.96	---	6.0E-05	3.89	0.067	0.023	0.531	1.55	0.027	0.018	0.063	2.37	0.068	0.026	0.049	0.24	0.011	0.043	6.9E-04	2.26	*SPATS2L*
2	201308486	rs893357	3.4E-06	2.70	V	1.2E-04	2.60	0.103	0.052	0.009	2.97	0.095	0.031	NA	NA	NA	NA	0.042*	0.48	0.043	0.086	0.0029	1.73	*SPATS2L*
4	91619360	rs72661871	7.8E-06	2.87	---	1.6E-03	2.66	0.078	0.043	0.005	5.50	0.072	0.021	0.059	2.34	0.075	0.041	0.848	1.06	0.062	0.058	2.0E-04	2.03	*CCSER1*
4	91671916	rs72661895	6.4E-06	2.92	---	8.0E-04	2.87	0.079	0.042	0.007	5.13	0.070	0.021	0.080	2.21	0.074	0.041	0.874	1.06	0.038	0.036	8.9E-05	2.21	*CCSER1*
5	**108402140**	**rs4957796**	**9.7E-08**	**0.52**	**---**	**7.2E-05**	**0.52**	**0.104**	**0.187**	**0.003**	**0.44**	**0.077**	**0.185**	**0.031**	**0.60**	**0.124**	**0.197**	**0.122**	**0.70**	**0.129**	**0.173**	**5.6E-08**	**0.56**	***FER***
5	108406299	rs975056	3.3E-07	0.56	VP	1.1E-03	0.61	0.149	0.227	0.001	0.44	0.095	0.227	0.016	0.58	0.145	0.234	0.239	0.79	0.183	0.217	4.6E-07	0.61	*FER*
5	**108417332**	**rs62375529**	**7.5E-07**	**0.56**	**---**	**1.3E-03**	**0.60**	**0.124**	**0.197**	**0.001**	**0.42**	**0.079**	**0.198**	**0.021**	**0.58**	**0.135**	**0.210**	**0.123**	**0.72**	**0.151**	**0.193**	**3.7E-07**	**0.59**	***FER***
5	134514728	rs553438	2.2E-05	0.63	---	9.6E-05	0.57	0.183	0.267	0.249	0.73	0.176	0.222	0.104	0.71	0.217	0.266	0.503	1.13	0.269	0.249	1.1E-03	0.74	intergenic
5	134532593	rs639405	8.0E-06	0.62	---	1.3E-04	0.58	0.201	0.283	0.115	0.67	0.180	0.242	0.069	0.69	0.231	0.282	0.798	0.95	0.234	0.245	1.6E-04	1.42	intergenic
6	**103810003**	**rs79423885**	**8.1E-06**	**2.05**	**---**	**6.9E-03**	**1.81**	**0.117**	**0.069**	**0.007**	**2.87**	**0.128**	**0.059**	**0.011**	**2.15**	**0.127**	**0.067**	**0.045**	**1.59**	**0.131**	**0.079**	**1.5E-06**	**1.89**	**intergenic**
6	103813490	rs77054842	7.3E-06	2.06	---	6.9E-03	1.81	0.117	0.070	0.007	2.87	0.128	0.059	0.010	2.17	0.128	0.067	NA	NA	NA	NA	NA	NA	intergenic
7	83635586	rs4732529	1.6E-06	1.78	VP	1.2E-04	1.86	0.230	0.161	0.033	1.79	0.236	0.161	0.047	1.60	0.210	0.147	NA	NA	NA	NA	NA	NA	*SEMA3A*
7	83670129	rs76881522	6.2E-06	1.77	---	3.1E-05	2.04	0.205	0.131	0.056	1.72	0.196	0.129	0.237	1.34	0.165	0.132	0.960	0.99	0.145	0.147	9.0E-05	1.54	*SEMA3A*
7	123248738	rs35947027	7.6E-06	1.54	GVP	3.6E-04	1.61	0.365	0.281	0.346	1.22	0.345	0.301	0.005	1.72	0.370	0.276	0.133	0.75	0.247	0.297	1.0E-03	1.33	*ASB15*
8	**19554995**	**rs12114790**	**6.4E-05**	**1.43**	**VP**	**5.0E-04**	**1.53**	**0.466**	**0.368**	**0.160**	**1.32**	**0.439**	**0.371**	**0.114**	**1.34**	**0.460**	**0.410**	**0.171**	**1.23**	**0.462**	**0.404**	**3.6E-05**	**1.38**	***CSGALNACT1***
8	19574243	rs10095344	1.0E-05	1.50	---	7.9E-04	1.51	0.445	0.354	0.054	1.48	0.462	0.367	0.032	1.47	0.462	0.395	NA	NA	NA	NA	NA	NA	*CSGALNACT1*
13	38969457	rs17057959	1.5E-06	1.78	---	1.2E-04	1.84	0.228	0.150	0.259	1.38	0.176	0.133	0.004	1.97	0.249	0.159	0.169	0.74	0.151	0.194	4.1E-04	1.45	intergenic
13	39102184	rs9566343	1.4E-06	1.71	GVP	7.1E-04	1.63	0.266	0.191	0.035	1.78	0.250	0.178	0.006	1.85	0.260	0.159	0.444	0.84	0.188	0.219	7.1E-05	1.48	intergenic

Supplementary Table 4. Top associations (loci with P < $1x10^{-5}$) after the genome-wide meta-analysis of 28 day survival among patients with sepsis caused by pneumonia or abdominal infections (additive model). Results shown for GenOSept (G), VASST (V), and PROWESS (P) discovery sets are based on imputed data (except where indicated), whereas additional GAinS results are based on directly genotyped data. Only the SNP with the most significant P-value per locus and those that were included in the additional GAinS genotyping are shown (more than one SNP per association peak was genotyped when possible). MAF=minor allele frequency; OR=odds ratio. NA denotes missing data.

Chr	Position	SNP id	Discovery Meta P	Discovery Meta OR	Directly genotyped	GenOSept P	GenOSept OR	GenOSept MAF cases	GenOSept MAF controls	VASST P	VASST OR	VASST MAF cases	VASST MAF controls	PROWESS P	PROWESS OR	PROWESS MAF cases	PROWESS MAF controls	Additional GAinS P	Additional GAinS OR	Additional GAinS cases MAF	Additional GAinS controls MAF	Combined P	Combined OR	Gene
2	49177601	rs7591064	9.5E-06	0.67	VP	7.7E-04	0.68	0.150	0.210	0.0188	0.62	0.143	0.217	0.082	0.71	0.178	0.219	NA	NA	NA	NA	NA	NA	intergenic
2	133396179	rs17324515	3.5E-05	0.74	GVP	8.9E-04	0.74	0.340	0.408	0.0083	0.65	0.348	0.457	0.395	0.87	0.395	0.414	0.387	1.11	0.423	0.399	1.8E-03	0.82	*LYPD1*
2	133426183	rs2709532	3.8E-06	0.71	---	1.9E-04	0.71	0.360	0.433	0.0035	0.61	0.347	0.468	0.343	0.85	0.405	0.426	0.416	1.10	0.442	0.424	1.2E-05	1.31	*LYPD1*
3	134815187	rs78690211	1.3E-06	1.99	---	4.6E-05	2.07	0.119	0.077	0.0921	1.85	0.092	0.060	0.041	1.87	0.122	0.085	0.402	0.82	0.069	0.082	2.3E-04	1.56	*EPHB1*
3	157311299	rs9876830	7.3E-06	1.42	VP	5.4E-04	1.43	0.370	0.312	0.0319	1.46	0.383	0.303	0.058	1.38	0.376	0.302	NA	NA	NA	NA	NA	NA	*C3orf55*
5	113387846	rs114618137	2.7E-06	1.80	---	5.9E-05	1.92	0.140	0.087	0.0452	1.76	0.132	0.083	0.121	1.53	0.129	0.100	NA	NA	NA	NA	NA	NA	lincRNA
6	163602261	rs942635	6.9E-06	1.54	---	2.6E-04	1.55	0.219	0.161	0.0082	1.82	0.202	0.129	0.277	1.28	0.168	0.132	0.565	0.90	0.145	0.152	2.1E-04	1.37	*PACRG*
6	163603305	rs2763993	4.6E-05	1.49	---	1.1E-03	1.48	0.200	0.148	0.0089	1.88	0.181	0.112	0.381	1.23	0.150	0.120	0.949	1.01	0.160	0.154	3.8E-04	1.35	*PACRG*
7	83635586	rs4732529	1.8E-06	1.59	VP	8.2E-04	1.52	0.206	0.160	0.0333	1.59	0.217	0.154	0.006	1.79	0.202	0.128	NA	NA	NA	NA	NA	NA	*SEMA3A*
7	83670129	rs76881522	1.4E-05	1.56	---	4.3E-04	1.58	0.179	0.130	0.0418	1.60	0.183	0.126	0.111	1.44	0.151	0.115	0.906	0.98	0.130	0.132	1.9E-04	1.39	*SEMA3A*
10	95820702	rs112692056	3.3E-06	2.20	---	1.7E-05	2.58	0.086	0.046	0.1768	1.61	0.080	0.054	0.097	1.93	0.068	0.047	NA	NA	NA	NA	NA	NA	*PLCE1*
13	27422997	rs74438932	1.1E-06	1.90	---	4.1E-05	2.04	0.111	0.068	0.0102	1.91	0.157	0.087	0.279	1.43	0.077	0.065	NA	NA	NA	NA	NA	NA	intergenic
17	67665781	rs6501341	3.5E-06	1.98	G	2.2E-04	2.04	0.099	0.063	0.0223	2.03	0.107	0.057	0.098	1.76	0.076	0.051	0.814	1.05	0.084	0.078	8E-05	1.61	AC003051.1
18	51427254	rs117914209	6.5E-06	2.96	---	5.5E-05	4.10	0.040	0.020	0.0901	2.37	0.046	0.024	0.090	2.10	0.049	0.028	NA	NA	NA	NA	NA	NA	intergenic
21	33704100	rs2096460	8.1E-06	0.61	---	8.3E-04	0.63	0.080	0.124	0.1725	0.69	0.104	0.133	0.005	0.50	0.083	0.149	0.781	0.95	0.121	0.129	6.5E-05	0.69	*URB1*

Supplementary Table 5. Multivariate Cox regression model for the effect of FER rs4957796 genotype on 28-day survival in patients with sepsis due to pneumonia, stratified by cohort.

Variable	Hazard Ratio (95% CI)	Wald test P-value‡
Genotype†	0.56 (0.45-0.69)	5.0×10^{-8}
Age (years)§	1.03 (1.02-1.04)	7.0×10^{-17}

LR test $\chi^2 = 111$; †Additive genotype model; §Continuous parameter. LR test (after adjustment for age and stratification by cohort) $p = 3.4 \times 10^{-9}$

Supplementary Table 6. Extended Cox regression model for the effect of *FER* rs4957796 genotype on survival to 6 months in patients with sepsis due to pneumonia in the GenOSept and GAinS cohorts, with genotype fitted as a time-varying covariate.

Variable	Hazard Ratio (95% CI)	Wald test P-value‡
Genotype†	0.64 (0.49-0.83)	0.001
Age (years)§	1.04 (1.02-1.04)	7.4×10^{-23}
Genotype-time interaction	1.01 (1.00-1.01)	0.003

LR test $\chi^2 = 124.2$; †Additive genotype model; §Continuous parameter

Supplementary Table 7. 28-day mortality in separate cohorts of patients with sepsis due to pneumonia according to *FER* rs4957796 SNP.

	Total N	**Died (within 28 days)**	**Survived**
GenOSept and GAinS (imputed discovery)			
CC	29	1 (3.4%)	28 (96.6%)
TC	285	33 (11.6%)	252 (88.4%)
TT	721	151 (20.9%)	570 (79.1%)
Total	1035	185 (17.9%)	850 (82.1%)
VASST (imputed discovery)			
CC	7	0 (0%)	7 (100%)
TC	49	11 (22.4%)	38 (77.6%)
TT	161	63 (39.1%)	98 (60.9%)
Total	217	74 (34.1%)	143 (65.9%)
PROWESS (imputed discovery)			
CC	10	2 (20.0%)	8 (80.0%)
TC	84	21 (25.0%)	63 (75.0%)
TT	207	77 (37.2%)	130 (62.8%)
Total	301	100 (33.2%)	201 (66.8%)
Additional GAinS (directly genotyped)			
CC	17	3 (17.6%)	14 (82.4%)
TC	140	20 (14.3%)	120 (85.7%)
TT	368	78 (21.2%)	290 (78.8%)
Total	525	101 (19.2%)	424 (80.8%)
All cohorts combined			
CC	63	6 (9.5%)	57 (90.5%)
TC	558	85 (15.2%)	473 (84.8 %)
TT	1457	369 (25.3%)	1088 (74.7%)
Total	2078	460 (22.1%)	1618 (77.9%)

Supplementary Table 8. *FER* rs4957796 association in patients with sepsis caused by microbiologically proven bacterial pneumonia. MAF= minor allele frequency; OR= odds ratio.

Cohort		**Pneumonia**	**Bacterial (gram negative and gram positive combined) Pneumonia**
GenOSept/GAinS (imputed)	N MAF non-survivors MAF survivors P OR	1035 0.104 0.187 **$7.2x10^{-5}$** 0.52	479 (46.3%) 0.095 0.193 **0.009** 0.50
VASST (imputed)	N MAF non-survivors MAF survivors P OR	217 0.077 0.185 **0.003** 0.44	136 (62.7%) 0.069 0.217 **0.003** 0.38
PROWESS (imputed)	N MAF non-survivors MAF survivors P OR	301 0.124 0.197 **0.031** 0.60	170 (56.5%) 0.119 0.201 0.102 0.60
Additional GAinS (directly genotyped)	N MAF non-survivors MAF survivors P OR	525 0.129 0.173 0.122 0.70	170 (32.4%) 0.083 0.183 **0.047** 0.40

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